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Effect of Seed Pretreatment by Magnetic Field on the Sensitivity of Maize Seedlings to Ambient Ultraviolet Radiation (280-400 nm)

Sunita Kataria, Lokesh Baghel, K.N.Guruprasad

ABSTRACT: *The effect of seed pretreatment with static magnetic field (SMF) of 200 mT for 1 h on the impact of ambient UV stress was tested using maize (JM 216) seeds. The SMF pre-treated and untreated seeds were sown in plastic nursery bags (34 cm H x 34 cm B; filled with mixture of sand, soil and manure -1:2:1) placed in metal mesh cages covered with polyester filters that cut off UV-B (<300 nm), UV-A/B (<400 nm) radiations, transmitted all the UV (280-400 nm) or without filters. The results of solar UV exclusion suggested that ambient UV caused reduction in growth and photosynthetic performance of maize seedlings. Whereas SMF-pretreatment increased the plant height, leaf area, biomass accumulation and improved the PSII efficiency and rate of photosynthesis under even in presence of solar UV components as well as under exclusion of solar UV-B and UV-A/B as compared to untreated seeds. The plants emerged from SMF pre-treated seeds also showed significant enhancement in nitrate reductase activity as compared to untreated seeds. Comparing SMF-treated groups with their respective untreated group, the remedial effects of SMF were found to be more striking on overall growth, nitrogen fixation and photosynthetic performance even in presence of solar UV stress. It indicates that magnetopriming of dry seeds of maize can be effectively used as a pre-sowing treatment for mitigating adverse effects of UV stress. Our results suggest that SMF seed pretreatment can be used in agriculture to better growth and improve the yield under the ambient UV stress.*

Key Words: *Static magnetic field; PS II; Photosynthesis; UV-B radiation.*

INTRODUCTION

Due to the concern on the depletion of the stratospheric ozone layer plant response to UV-B radiation was extensively studied [1]. Plants are unavoidably exposed to UV radiation, as they require sunlight to carry out photosynthesis, and have therefore evolved protection and repairing mechanisms resulting in UV acclimation. As a significant potential environmental stress in natural ecosystems and agriculture, UV-B irradiation decreased growth and yields of a majority of agricultural crops. The decreases largely resulted from changes in leaf physiology and function [2-3] such as depressed leaf photosynthesis, changed leaf ultrastructure and anatomy [4-6] and destroyed cell DNA, protein and lipids by increased oxidative stress [2]. In photosynthetic organisms, increased UV and ambient UV radiation may cause critical impairments in the photosynthetic apparatus leading to pigment degradation, photoinhibition and reductions in quantum yield, net photosynthesis and Calvin cycle enzyme activity, together with DNA and oxidative damage [2, 6, 7].

As two parts of radiation biology, magnetic fields (MF) treatment had positive effect on plant growth and development [8-9] whereas ambient UV-B radiation had negative effect on plant growth and development [7]. Both magnetic treatment and UV-B radiation acted on the cell membrane, changed plant photosynthetic function and enzyme activity. However, there has been no report on plant response to the combination of seed MF-pretreatment and ambient UV (280-400 nm) radiation.

Magnetic fields were used widely as presowing seed treatments to increase seed vigor, seedling growth and yield [9-10]. Previous studies indicated that suitable magnetic treatment increased the absorption and assimilation of nutrients [11] and ameliorated photosynthetic activities [8-9]. Many studies found that MF-pretreatment could alleviate the inhibitory effect of salt [12], heat and drought stress [13-14]; enhanced its saline-alkali tolerance [15],

* School of Life Science, Devi Ahilya Vishwavidyalaya, Khandwa Road, Indore (M.P.)-452001, India, *E-mail- sunitakataria@hotmail.com*

and delayed the senescence process [16]. In this study, we used maize (*Zea mays)* var. JM 216 as experimental material to study the effect of seed pretreatment by static magnetic field (SMF) of 200 mT for 1h on sensitivity of maize seedlings to ambient UV (280-400 nm) radiation. Maize is the third most important crop worldwide and is sensitive to increased UV-B [17] and ambient UV [18] radiation. The aim of the present study was to evaluate the possible contribution of pre-sowing treatment of SMF to alleviate the damaging effect of ambient UV stress on the maize seedlings. For this reason, the present study was intended to assess the impact of current level of solar UV by the exclusion of UV-B and UV-A/B after magnetopriming of the maize seeds.

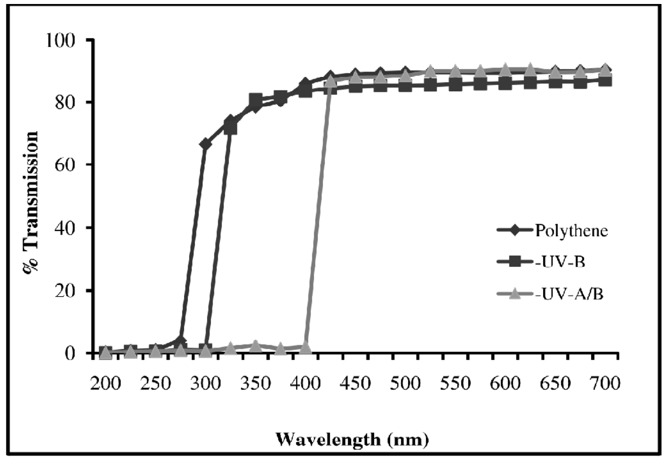

Figure 1: Transmission spectra of UV cut off filters and polyethene filter used for raising maize seedlings under ambient field conditions

MATERIALS AND METHODS

Plant material and growth conditions

The experiments were conducted on the terrace of the School of Life Sciences, Devi Ahilya University, Indore, India (latitude 22.48°N) during October 2014 to January 2015 under the ambient environmental conditions. The seeds of maize (*Zea mays* var. JM 216) were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara (M.P.), India and treated with recommended fungicides viz. Bevistin and Diathane Mat 2 g/kg seeds before sowing. The SMF pre-treated and untreated seeds of maize were sown in plastic nursery bags (34 cm H x 34 cm B; filled with mixture of sand, soil and manure -1:2:1). Thereafter the bags were immediately placed in metal mesh cages 1.2 m length x 0.91 m width x 0.91 m height covered with polyester filters (Garware polyesters Ltd., Mumbai) that cut off UV-B (<315 nm) and UV-A/B (<400 nm) radiations. Filter control (FC) plants were grown under a polyethylene filter which transmits all the ambient solar radiations including the UV-A/B components, or in open field without any filter exposed to ambient solar radiation (open control OC). There were three metal mesh cages for each filter control/open control, -UV-B and -UV-A/B treatment.

The transmission characters of these filters are given in Figure 1. The transmission characteristics of filters did not change during the experimental period and these filters did not emit fluorescence in visible regions. The filters were erected at the time of germination and were maintained until maturity. The bottom sides of all the frames were left uncovered to allow normal ventilation. The frames received full solar radiation for most of the day without any shading. Irrigation was given as and when required for optimal growth of the crop. Temperatures both inside and outside each enclosure were monitored daily using max/min thermometer. The average temperature at outside raised from 25 to 32°C during the growing period. Due to the passive ventilation system no significant increase in the temperature was measured inside the chambers compared with ambient air

Magnetic treatment

An electromagnetic field generator "Testron EM- 20" with variable horizontal magnetic field strength (50–500 mT) with a gap of 5 cm between pole pieces was fabricated [for the detail of the instrument refer to Vashisth and Nagarajan [19]. Maize seeds were exposed to a static magnetic field of 200 mT (1 h) in a cylindrical shaped sample holder of 42 cm^3 capacity, made from a non-magnetic thin transparent plastic sheet. For SMF treatment, hundred visibly sound, mature healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field. By regulating the current in the coils of the electromagnet, the required strength of the magnetic field was obtained. A Gauss meter was used to measure the strength of the magnetic field between the poles.

Radiation measurements

Absolute solar irradiance with or without UV-B or UV-A/B was measured using a radiometer (Solar light PMA2100, Glenside, PA., U.S.A). The ambient solar irradiance during experimental period at midday was 1450 µmol m^{-2} s^{-1}, under -UV-A/B filters the loss in light intensity at midday was 11.8% (1280 µmol m^{-2} s^{-1}), under -UV-B filters was 12.5% (1270 μmol m^{-2} s^{-1}) and under polythene filter transmissible to UV (filter control) was 4.2% (1390 μmol m^{-2} s^{-1}).

Growth Data collection and analysis

Plants were sampled randomly in triplicates from all the treatments at 50 days after the emergence of seedlings (DAE). Plant height was measured from the ground level to the raised leaf-tip with meter rule. After 50 DAE the sampled maize plants from the plastic bags were harvested and taken to the laboratory. The total biomass was determined on a top-loading balance. For total biomass accumulation, the above ground parts of the plants and were oven-dried at 105 °C for 24 h to constant weight. Area of leaves was measured using portable laser leaf area meter CID-202 scanning planimeter (CID Inc., USA).

Pigment Analysis

The chlorophyll (Chl) content was determined by dimethyl sulfoxide (DMSO) method as described by Hiscox and Israelstam [20]. Equations of Wellburn and Lichtenthaler [21] were used to calculate the Chl concentrations in leaves of 50 day old plants of maize.

Chlorophyll Fluorescence

Chlorophyll *a* (Chl *a*) fluorescence induction kinetics of dark-adapted (30 min) leaves (topmost fully opened leaves at 50 DAE) of maize was measured using a Handy PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments, King's Lynn, Norfolk, UK). Excitation light of 650 nm (peak wavelength) from array of six light-emitting diodes focused on a 4 mm diameter spot on the leaf surface to provide a homogenous illumination. The light intensity reaching the leaf was 600 W/m^2 (3200 μE/m^2/sec) which was sufficient to generate maximal fluorescence for all treatments. Data were recorded for 1 s with 12-bit resolution; the data acquisition was for every 10 ms for the first 2 ms and every 1 ms thereafter [22]. Chlorophyll fluorescence characteristics such as maximum potential quantum yield of photosynthesis ratio (F_v/F_m) ratio and quantum yield of electron transport (φEo=ETo/ABS) were measured. The performance index on an absorption basis (PI_{ABS}), reflecting the performance of the overall energy flow was also calculated.

Gas exchange measurements

Net photosynthesis (*Pn*, μmol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) was measured by a portable photosynthetic system (Li- 6200, LI-COR Inc., Lincoln, Nebraska, Serial No. PPS 1332 USA) in intact plants emerged after SMF treatment grown under normal sunlight or UV excluded sun light under field conditions at midday between 11.00 to 12:00 at 50 DAE. Photosynthetic measurements were made with fully expanded top most leaves in each variety from each treatment under ambient temperature and CO_2 concentration, on clear days, photosynthetic photon flux density (PPFD) was 1300-1600 μmol m^{-2} s^{-1}, air flow (500 μmol s^{-1}) and CO_2 concentration (350-380 ppm).

Determination of nitrate reductase (NR) activity

Nitrate reductase (E.C. 1.6.6.1) activity in the leaves of maize plants at 50 DAE and it was determined by the intact tissue assay method of Jaworski [23].

STATISTICAL ANALYSIS

All the data are presented in triplicates, five plants from each replica were taken for the recording of all parameters studied (n=3). The data are expressed as means ± S.E.

RESULTS AND DISCUSSION

Ambient and enhanced UV-B radiations affect the physiological process, growth and development of plants [24-26]. Previous reports suggested that maize is sensitive to both ambient UV-B [18] and enhanced UV-B [17]. The results of present study showed that exclusion of ambient UV radiation from solar spectrum significantly enhanced the growth and photosynthesis of maize plants. The exclusion of solar UV-A/B showed that ambient UV components caused 48% reduction in plant height, 49% in plant biomass and 125% in leaf area under filter/open control (Fig. 2A). On the other hand treatment of maize seeds with SMF of 200 mT for 1h caused significant promotion in all the measured growth parameters as compared to untreated seeds even in the presence of ambient UV stress (OC and FC) (Fig. 2A,B,C). Most altered parameters by SMF pretreatment under ambient UV stress were plant height (30%), leaf area (45%) and total biomass accumulation (46%) (Fig. 2A,B,C). However without solar UV components (–UV-B and –UV-A/B) the SMF treatment to the seeds did not cause any significant change in the growth parameters as compared to the untreated seeds (Fig. 2A,B,C).

In the present study it was observed that exclusion of solar ultraviolet radiations caused a significant increase in total chlorophyll (Chl) content due to the significant increases in Chl *b* rather than Chl *a* (Fig.

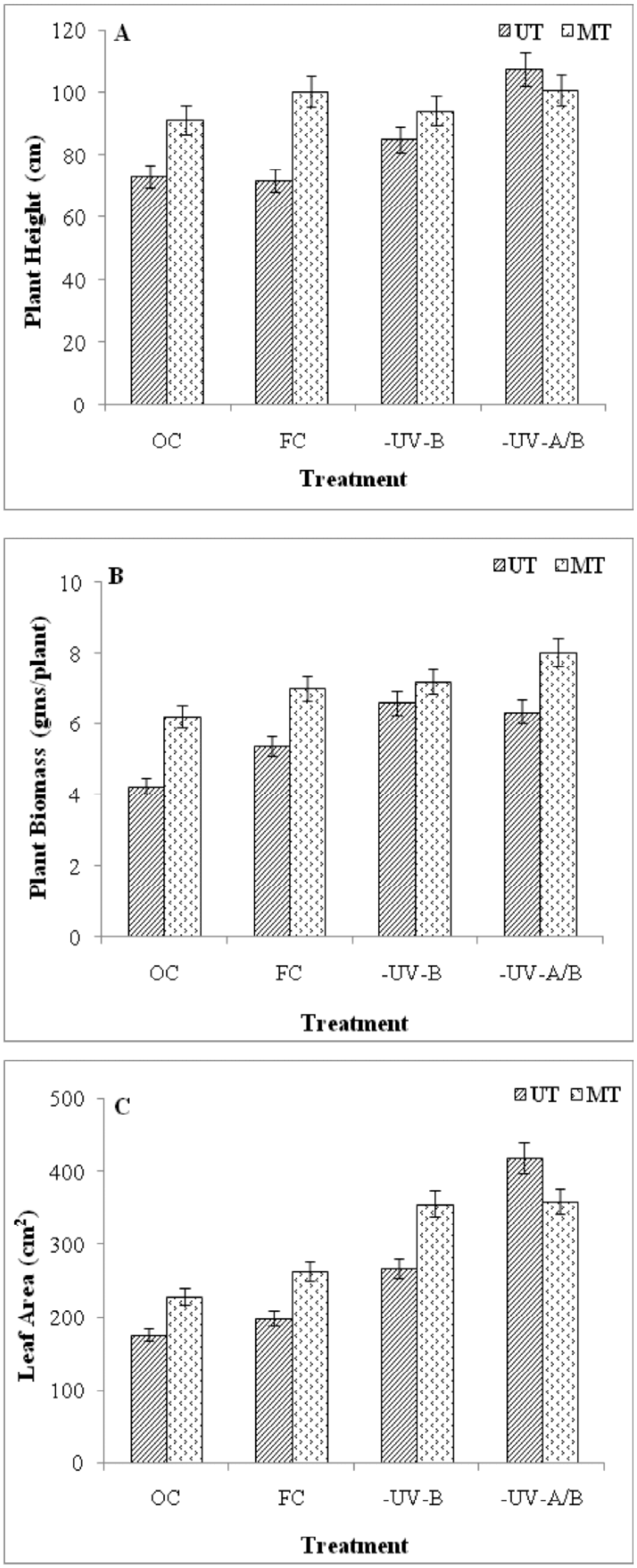

Figure 2: Effect of ambient UV and SMF-pretreatment on plant height (A), above ground biomass (B) and leaf area (C) of maize at 50 days after emergence of the seedlings. The vertical bar indicates ±SE for mean

3A,B,C) as compared to ambient controls (OC and FC). It indicates that ambient UV decreased the *Chl* content however; the SMF treatment to the maize seeds, reduced the damage to the chloroplasts to some extent and it increased the chlorophyll content significantly as compared to untreated seeds (Fig. 3A,B,C). 23% of increase was recorded in total chlorophyll content after 200 mT SMF exposure under ambient control conditions (Fig. 3C). Enhancement in the level of chlorophyll *b* (134%) was higher than chlorophyll *a* (25%) after SMF pretreatment, due to this chlorophyll *a/b* ratio was decreased after magnetic field treatment (57%) (Fig. 3A,B,D).

Chlorophyll fluorescence, an important indicator of reversible and irreversible changes of PSII reaction centers in plants, was characterized as sensitive general biomarker to UV exposure [27]. The SMF pre-treatment significantly enhanced the maximum quantum efficiency of PSII (Fv/Fm) (Fig. 4A) and the quantum yield of electron transport (φEo = ETo/ABS), were also increased in plants that emerged from magnetically treated seeds as compared to untreated seeds in the presence of solar UV components (Fig.4B). However without solar UV components (–UV-B and –UV-A/B) the SMF treatment to the seeds did not cause any significant change in these parameters as compared to the untreated seeds (Fig.4B).

On the other hand exclusion of solar UV components also enhanced all these parameters in untreated seeds of maize as compared to the ambient control which received both UV-B and UV-A radiations. Results on chlorophyll *a* fluorescence reveals that leaves of maize plants that emerged from magnetically treated seeds or from UV excluded plants had higher reducing power with higher quantum yield of electron transport (Fig. 4A,B).

The most sensitive parameters calculated by the equations of the JIP test is performance index (PI), which is an indicator of sample vitality. This was significantly enhanced by the SMF pre-treatment and exclusion of solar UV components (Fig. 4C). PI is a good indicator about the vitality of the photosynthetic organisms where the decreased vitality is expressed by low PI_{ABS} index values, like under ambient UV stress condition (OC and FC). The PI is a function of three independent functional steps of photosynthesis, i.e. RC/ABS, φPo and ψo [28]. PI_{ABS} was enhanced by 42% and 53% after the pre-treatment of SMF under ambient control and filter control conditions respectively. Exclusion of UV-B and UV-A/B also increases PI_{ABS} by 70% and 30% respectively as compared to the ambient untreated controls (Fig. 4C).

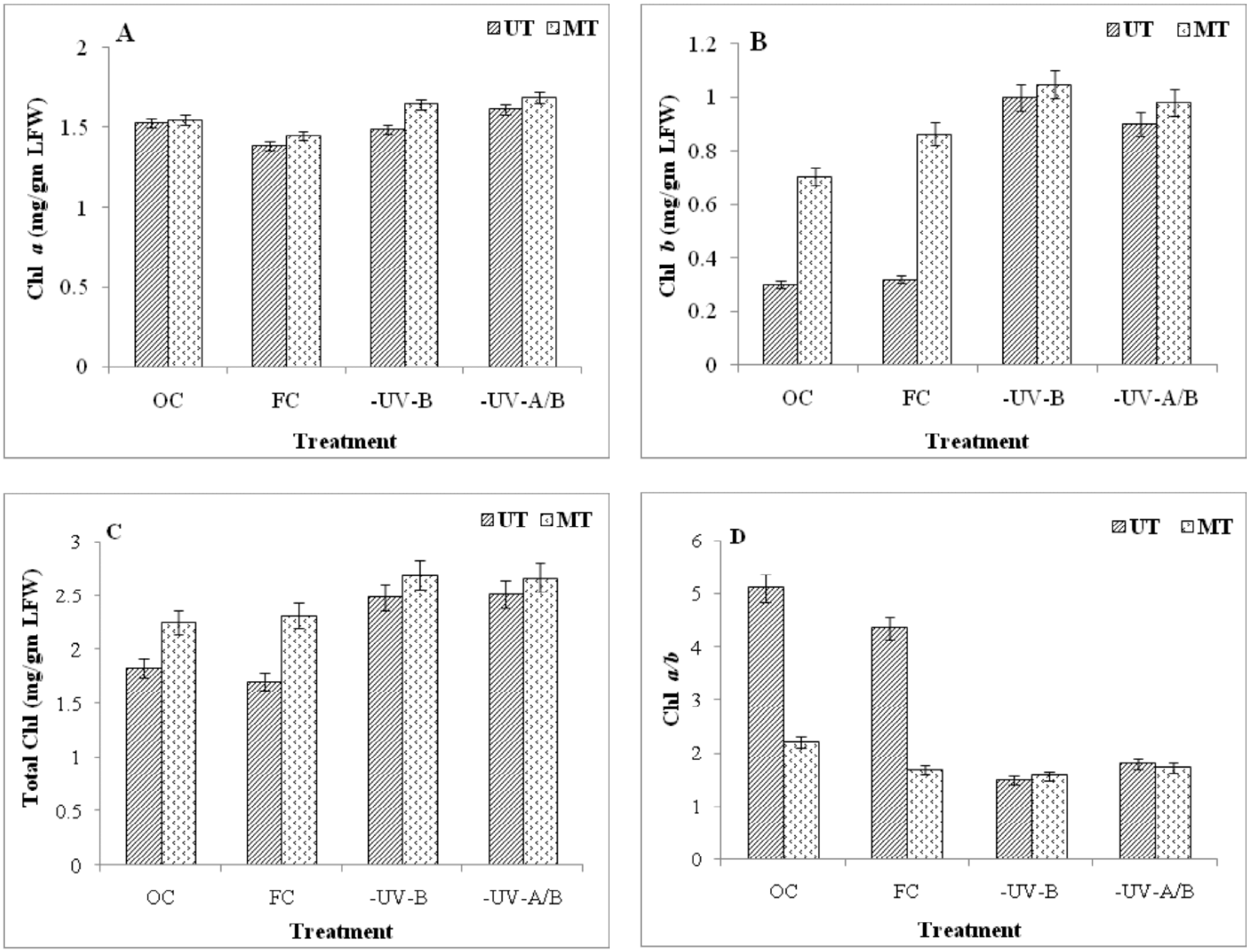

Figure 3: Effect of ambient UV and SMF-pretreatment on chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C) and chl *a/b* (D) in leaves of maize at 50 days after emergence of the seedlings. The vertical bar indicates ±SE for mean

Solar UV exclusion caused significant enhancement in NR activity as compared to the control plants grown under ambient and filter control treatments. On the other hand the plants that emerged after SMF treatment also showed higher activity of NR in all the treatments like ambient and filter controls as well as in UV excluded plants also as compared to their untreated seeds (Fig. 4D).

Exclusion of solar UV components significantly enhanced the net rate of photosynthesis in terms of CO_2 absorbed and this was accompanied by an increase in the stomatal conductance (Fig. 4E,F). It indicates that presence of solar UV components suppress the fixation of CO_2. The increase of 52% in net photosynthesis (*Pn*) was recorded in the plants that emerged from magnetically treated seeds as compared to untreated seeds under ambient UV stress (OC) (Fig. 4F).

Ambient UV (280-400 nm) irradiation significantly decreased growth, development and biomass accumulation along with reduced efficiency of PSII, PI_{ABS} and rate of photosynthesis of maize seedling in the present study, which was accorded with previous study [18]. While the combination of seed SMF-pretreatment with ambient UV radiation increased the leaf growth, above ground biomass and leaf area as compared to untreated seeds. Thus SMF pretreatment protects the maize plants from the adverse effects of ambient UV stress. Some previous studies demonstrated that MF pretreatment could ameliorate inhibition of other stresses. The inhibitory effect of heat stress on cress (*Lepidium sativum*) seedlings was alleviated by MF-pretreatment [13]. MF-pretreatment with 0.2–0.3 T for 10 min enhanced saline-alkali tolerance of *Leymus chinensis* seedlings [29] and drought tolerance of wheat seedlings [30].

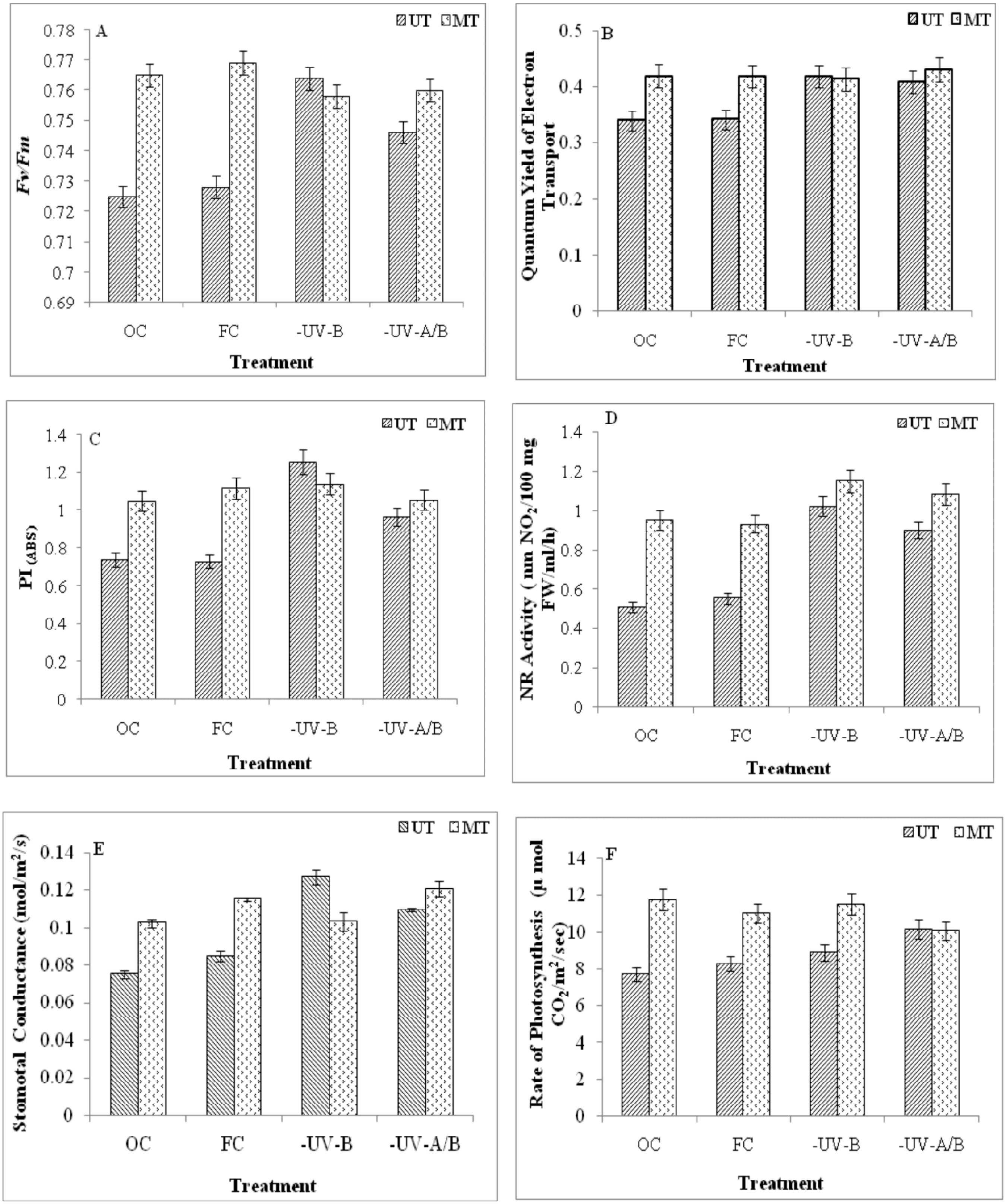

Figure 4: Effect of ambient UV and SMF-pretreatment on the maximum potential quantum yield of photosynthesis (Fv/Fm) (A), quantum yield of electron transport (φEo = ETo/ABS) (B), Performance index (PI_{ABS}) (C), NR activity (D), stomatal conductance (E) and net rate of photosynthesis (F) of maize at 50 days after emergence of the seedlings. The vertical bar indicates ±SE for mean

Cakmak et al. [31] investigated the effects of low static magnetic field (4 or 7mT) on seedling growth of bean or wheat seeds in different osmotic conditions and reported significant enhancement in early growth and dry biomass accumulation of bean and wheat seeds. Also, treatment of lentil seeds with magnetic fields increased mean germination time, seedling length and seedling dry weight [32].

Our results suggested that magnetic field might be involved in the improvement of maize plant growth due to the better harvesting of light, higher activity of nitrate reductase and higher rate of photosynthesis. On the other hand similar kind of results was obtained by the exclusion of solar UV-B and UV-A/B which indicated the parallelism between the two effects. Both enhanced the growth of the plants, biomass accumulation, nitrate reductase activity and photosynthetic performance of maize plants.

In conclusion, our results showed that the growth of maize was influenced after pre-sowing treatment of their seeds with static magnetic field of 200 mT for 1 h. The exclusion of solar UV-B and UV-A/B indicated that ambient UV caused stress and reduced the growth and photosynthetic performance in maize plants. On the other hand when the plants emerged from SMF pre-treated seeds, they showed significant increase in plant height, leaf area, biomass accumulation, nitrate reductase activity and improved the efficiency of PS II and the rate of photosynthesis under ambient UV (280-400 nm) stress as well as under exclusion of solar UV as compared to untreated seeds of maize. Thus pre-sowing treatment of SMF can be effectively used for alleviation of adverse effect of UV stress in crop plants. These increases could have important economic implication and be beneficial especially for farmers for increased yield under field conditions. Using magnetic field treatment could be a promising technique for agricultural improvements although extensive research is required at field level.

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